THE BIOCHEMISTRY OF INSECTICIDE RESISTANT HOPPER RACES

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ABSTRACT

Many species of pest insects are able to establish highly specialized biochemical races when subjected to a continuous insecticide selection pressure. This evolutionary process leads to insecticide resistance, a phenomenon of great practical importance. The newly acquired biochemical features (insensitive target enzyme for insecticidal carbamates, enzymatic degradation for organophosphates) of resistant plant- and leafhopper populations are rather well understood and suitable for the purpose of monitoring field populations for resistant biotypes.

INTRODUCTION

The biochemical mechanisms by which insecticide resistant arthropod populations in general (Oppenoorth & Welling 1976), and hoppers in particular (Hama 1980) avoid intoxication and death are now well understood. Two of them are of special importance: (1) Target insensitivity towards, and (2) enzymatic degradation of insecticides.

The evolutionary process of 'acquiring' a modified target and/or a potent degradation enzyme is driven by directed selection through insecticides. The resulting biotypes not only minimize the effects of plant protection measures, but also reduce the lifetime of insecticides, a serious problem for an industry facing ever increasing costs for research and development.

ACETYLCHOLINESTERASE

Insecticidal organophosphates and carbamates kill insects by inhibiting acetylcholinesterase (AChE), a vital enzyme in the insect's nervous system. It has drawn the utmost attention of biochemists engaged in mode of action and resistance studies.

The following arthropod species are now known to have a resistance mechanism based at least partly on insensitive AChE's:

Tetranychus urticae Koch	(Smissaert 1964)
Boophilus microplus Can.	(Lee & Batham 1966)
Nephotettix cincticeps Uhl.	(Hama & Iwata 1971)
Musca domestica L.	(Tripathi & O'Brien 1973)
Spodoptera littoralis Boisd.	(Zaazou et al. 1973)
Anopheles albimanus Wied.	(Ayad & Georghiou 1975)
Nilaparvata lugens Stål	(Hama & Hosoda 1982)

Nephotettix cincticeps Uhler

Insensitivity of AChE is the major mechanism prevailing in resistance towards aryl N-methylcarbamates, a group of insecticides widely used in Japan. It also plays a role in resistance against organophosphates of the O,O-dimethyl type, such as malathion, methyl parathion and fenitrothion. The modified enzyme can also be distinguished from its wild-type precursor by being more susceptible towards aryl N-propylcarbamates, and by having a lower affinity towards acetylthiocholine, the artificial substrate used in determining enzyme activities (Hama 1980). These and other findings by Hama and coworkers suggest qualitative changes at the hydrophobic site of the enzyme.

By measuring AChE activities in the presence of 6.7×10^{-4} M propoxur in single heads of green rice leafhoppers, Hama (1982) was able to establish frequency distributions (%) for AChE insensitivities within several strains of this species. As outlined in Figure 1, all individuals (100 %) of the susceptible strain S carried a sensitive AChE (= 0 % AChE in the presence of the inhibitor propoxur) whereas the measurements for the resistant strains Rns and RK pointed to an insensitive enzyme. The frequency distributions for the individuals of these strains were rather uniform and found to be between the 80-100 % AChE level. The results for the two F_1 generations obtained by crossing S with Rns and RK, respectively, and the correspondong backcrossings between F_1 and S with a clear segregation into two sensitivity phenotypes (1:1 ratio) indicate that the insensitivity of AChE associated with carbamate resistance is genetically controlled by a single, incompletely dominant factor.

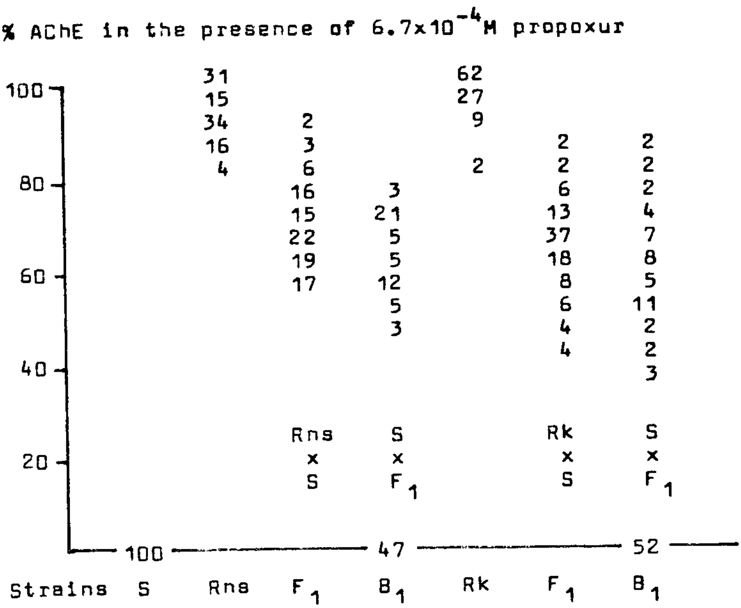

Fig. 1. Single head AChE sensitivity distributions (%):
Genetic experiments with 3 strains of Nephotettix cincticeps Uhl.

A first analysis of field populations from several parts of south-western Japan suggested site related differences (Hama et al. 1982). Strains from the islands of Honshu and Kyushu apparently developed AChE insensitivity in one step. The frequency distributions among the individuals belonging to the two or three phenotypes observed were compatible with the Hardy-Weinberg equilibrium, assuming AChE insensitivity being controlled by a single allele. On the island of Shikoku, however, populations were found with a two-fold AChE activity developing AChE insensitivity in two steps. Hama (1982) proposes a two loci/two mutations hypothesis in order to explain these recent findings.

Nilaparvata lugens Stål

Although the levels of insecticide resistance in the brown planthopper are still far below those observed for the green leafhopper, there is now evidence that Nilaparvata lugens is also capable of establishing biochemical races carrying an insensitive AChE (Hama & Hosoda 1982). Strain R, listed in Fig. 2, can serve as an example. A comparison with a susceptible counterpart S shows an extremely wide frequency distribution for AChE sensitivity. A laboratory selection with propoxur for six generations led to strain R6p, which exhibited a more distinct AChE sensitivity difference when compared with S. Another resistant strain (B), however, was not different from S, a finding which points to a second resistance mechanism: enzymatic degradation.

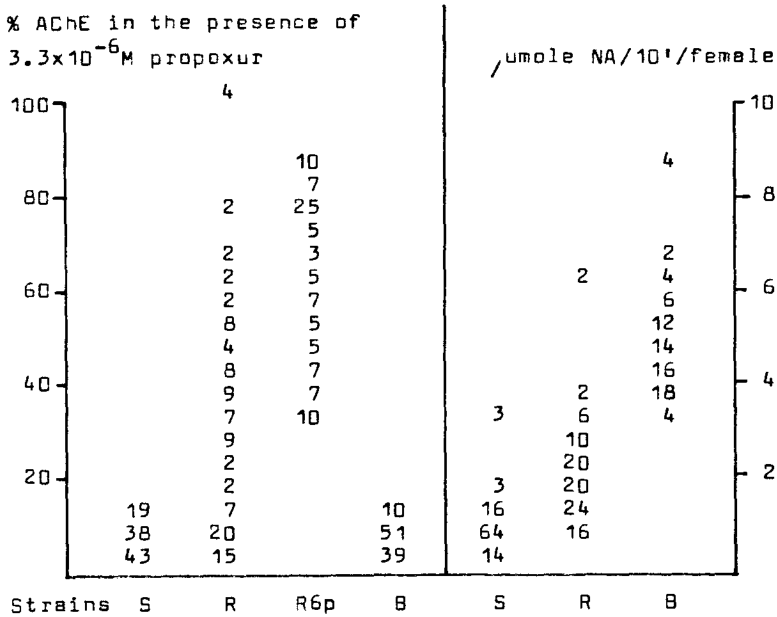

Fig. 2. Single head AChE sensitivity distribution (%, left hand side), and esterase activity distributions (determined with α-naphthylacetate, right hand side) of brown planthoppers, Nilaparvata lugens.

ALIESTERASES AND CARBOXYESTERASES

Organophosphate resistant Nephotettix cincticeps, Laodelphax striatellus (Ozaki 1969) and Nilaparvata lugens (Hama & Hosoda 1982) can be characterized by an abnormally high activity of aliesterases, hydrolytic enzymes detectable with naphthylacetate. Thus, the right hand part of Fig. 2 clearly indicates this elevated enzyme activity, particularly for strain B (mentioned above and said to have a normal, i.e. sensitive AChE). Furthermore, in several strains of brown planthoppers total aliesterase activities were closely related to LD50 values for malathion, fenitrothion and propoxur (Hama & Hosoda 1982, Table 1).

TABLE 1.

Relationship between LD50 values for three insecticides and total esterase activity (mean value 20 females) of six strains of Nilaparvata lugens. Nymphs of strain B were selected with BASSA insecticide (2-sec-butylphenyl methylcarbamate) for 50 generations in the laboratory.

Strain	Site of collection	Year	LD50 for females (µg/g) Malathion	Fenitrothion	Propoxur	µmole α-NA per 10 min/♀
TM	TSURUMAKI	1966	7.8	9.6	0.24	0.69 ± 0.09
S	TAKEHARA	1973	12.6	16	0.61	0.94 ± 0.21
R	DITO	1980	73	83	2.6	1.96 ± 0.32
TK	DITO	1979	120	96	3.7	2.91 ± 0.44
H	HACHIHONMATSU	1979	129	108	3.8	3.10 ± 0.21
B	TAKEHARA	1975	672	509	4.2	5.10 ± 0.75

Agar gel electrophoresis has been extremely helpful in studying the relationship between certain bands of aliesterase activity and carboxyesterase capable of degrading malathion. In both resistant Nephotettix cincticeps and Laodelphax striatellus carboxyesterase appeared to be very similar or identical to specific bands of aliesterase (see Hama 1980).

DISCUSSION

The rates by which insect AChE's react with different inhibitors are related to taxonomy. The inhibition properties are at least species specific, but often cover large taxonomic units (Voss 1981). Only if insect populations are subjected to a continuous selection pressure by organophosphates or carbamates, do phenotypes with an atypical target enzyme become predominant.

AChE insensitivity and/or aliesterase activity measurements in single field collected insects are a useful tool to (1) monitor populations for resistant phenotypes, (2) to predict the further development of a resistance situation, and (3) to establish sound strategies for appropriate countermeasures. From a technical point of view the necessary enzyme determinations can often be automated, as shown in Figures 3 and 4 for two strains of houseflies.

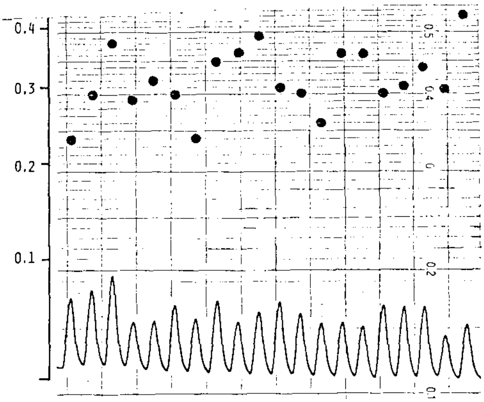

Fig. 3. AChE activities in 20 single heads of houseflies (susceptible laboratory strain). Dots show original activities (without inhibitor), peaks at the bottom remaining activities after adding 0.1 ppm dichlorvos to the automated assay system.

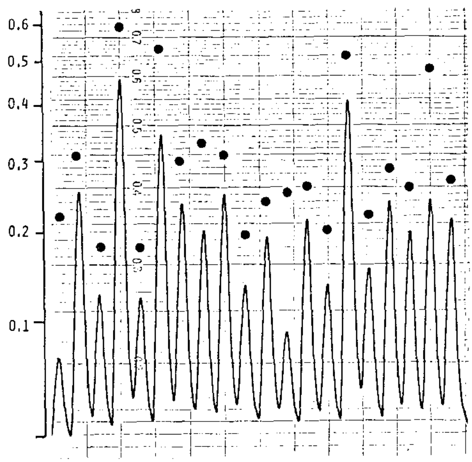

Fig. 4. AChE activities in 20 single heads of houseflies (a Swiss field strain). For explanation see Fig. 3.

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DISCUSSION

T.S. Robertson: In view of some of the contributions to the present workshop, what does Dr. Voss see as the future for insecticides in controlling leafhoppers and planthoppers, especially on rice?

Dr. Voss: Current research and development work on hopper insecticides aims at solving or improving two major problems:
(1) insecticide resistance and resurgence, and (2) insecticide selectivity as related to beneficial arthropods and fish.

The relative importance of these problems differs among the rice growing regions in Asia, and the attitudes in chemical insect control strongly depend on levels of economy and technology. In Japan, for example, a new selective growth-regulator type insecticide against Nilaparvata lugens may find its way to the market more easily than it does in developing countries where stemborers, hoppers and gallmidges may have to be controlled by a fast acting, non-selective and inexpensive compound. Also, the region-specific resistance problem the Japanese have with their green rice leafhoppers calls for specific solutions. Finally, fish toxicity is not of equal importance everywhere, but attempts generally to improve the situation rank high in present industrial research. In 5-10 years from now we may even have pyrethroids as rice insecticides.

Although considerable progress has been made in managing insect pests by using resistant rice varieties or applying various cultural methods, insecticides will continue to play a major role in controlling rice insects. This crop has to feed an ever-increasing number of people in overpopulated areas, and the assumption that we would not have to rely on plant-protection chemicals in the future, even the long-term future, appears to be unreasonable. This outlook, however, is accompanied by the statement that the research-oriented plant protection-industry accepts the challenge of integrated pest management.